Neural Grafting in the Mammalian CNS
A. Björklund and U. Stenevi, editors

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Transplantation of Tissues to the Cerebral Ventricles: Methodological Details and Rate of Graft Survival

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I. Introduction

The cerebral ventricles have several properties that suggest they are favorable sites for tissue transplantation. As a fluid-filled space immediately adjacent to many subcortical structures, the ventricular system may act as a conduit for the transport of neurohormones, and in fact contains many biologically active peptides (Wood, 1983). The ventricles also provide spaces into which tissues can be placed without damaging the brain parenchyma, and the cerebrospinal fluid may act as a nourishing medium for the initial maintenance of graft tissues prior to vascularization. Grafts in the ventricular system can be arranged to contact any of many periventricular structures (Table I).

It has long been recognized that various kinds of grafts frequently survive when transplanted to the brain even if rejected when transplanted to other parts of the body. This preferential survival of graft tissues in the brain and certain other locations, such as the anterior chamber of the eye, has been termed 'immunological privilege'. One of the earliest studies of tissue transplantation in the brain, by Murphy and Sturm (1923), concluded that a preferential survival of grafts in the brain occurred for tissues located entirely within the brain parenchyma. But, if the grafted tissues came into contact with the ventricular system they did not seem to survive. The conclusion

TABLE I

SOME STRUCTURES ADJACENT TO THE VENTRICULAR SYSTEM IN THE RAT

Caudate-putamen	Dorsal thalamus
Corpus callosum	Superior colliculus
Lateral septum	Cerebellar vermis
Stria terminalis	Locus coeruleus
Hippocampus	Hypothalamus
Fimbria, fornix	Nucleus reuniens
Medial habenula	Subfornical organ

that grafts contacting the cerebral ventricles do not enjoy immunological privilege was generally accepted by subsequent investigators (e.g. see Barker and Billingham, 1977).

The first investigators to recognize favorable properties of the cerebral ventricles as a site for transplantation were Rosenstein and Brightman (1978), who found that homologous (see Table III for definitions) grafts of superior cervical ganglia consistently survived in the fourth ventricle. Subsequently, intraventricular grafts of embryonic substantia nigra, adrenal medulla, spinal cord, hypothalamus, and other tissues have been found to survive and sometimes to alter the functioning of the brain of the host animal (Perlow et al., 1979; Freed et al., 1980, 1981; Freed, 1983; Gash and Sladek, 1980; Reier et al., 1983; Rosenstein and Brightman, 1978, 1979.)

II. Methods

II.1. Tissue preparation and dissection

The methods for dissection of embryonic brain tissues and other tissues have been discussed elsewhere (Freed, 1983; Rosenstein and Brightman, 1979; Seiger and Olson, 1977). It is important that tissues are prepared under clean conditions, although strict sterile procedure is not required. All materials used in the procedure (e.g. tools, glassware, plasticware and solutions) are sterile. The tissues to be transplanted, once obtained, must be cut into pieces small enough to fit comfortably into an 18-gauge spinal tap needle (a 20-gauge needle may also be used). In general, this results in a final volume of about 0.5 mm^3 per piece, although this varies to some degree depending on the consistency of the tissue. It is not desirable to cut the tissue into very small pieces. Several pieces of tissue (as many as six to eight) may be trans-

TABLE II

MATERIALS

Anesthetic composition:	Chloral hydrate (26.2 g); $MgSO_4 \cdot 7H_2O$ (13.1 g); dissolve in 100 ml distilled water. Add pentobarbital standard solution (100 ml); propylene glycol (292 ml); ethanol (76 ml); mix and add distilled water to a total volume of 770 ml. Administer 2.6–3.0 ml/kg, i.p.
Antibiotic:	Flo-Cillin (Bristol Laboratories, Syracuse, NY 13201). Administer 0.6 ml/kg per rat s.c.
Anesthetic for donors:	Ether, or a combination of 3 ml ketamine (Vetalar, Parke-Davis) and 7 ml xylazine (Cutter Labs., 20 mg/ml) Administer 1.5 mg/kg, i.m.
Needle holder:	No. 1272 Universal Holder, David Kopf Instruments, Tujunga, CA 91042.
Injection needle for method A:	Perfektum 7424 (N3324) thin wall, short Quincke Bevel 18-gauge × 3 in., Popper and Sons, Inc., New Hyde Park, NY 11040.
Injection needle for method B:	Perfektum 7533 (N1504) Tuohy Needle 18-gauge × 3 in., Popper and Sons, Inc., New Hyde Park, NY 11040.
Other materials required:	15-gauge needle for guide cannula 1 ml plastic disposable tuberculin syringes 25-gauge hypodermic needles Large syringes and needles 60 mm diameter sterile Petri plates Lactated Ringer's solution Dissecting instruments Surgical instruments Dissecting microscope Pasteur pipettes Stereotaxic instrument Dental drill, shaver Ethanol Gauze (surgical sponge) Gelfoam Wound clips Watch glasses

planted with a single needle penetration. Tissues are dissected and stored in lactated Ringer's solution at room temperature at all times. The tissues should be handled gently using Pasteur pipettes or by manipulating with a blunt instrument. Tissues must be used within, at most, 30 min after dissection.

II.2. Animal preparation

(i) Rats are anesthetized with a combination of chloral hydrate and pentobarbital (Table II), their heads are shaved, and they are mounted in a stereotaxic instrument in the Pellegrino et al. (1979) position, with the bite bar 5.0 mm above the interauricular line. (ii) The scalp is cleaned with ethanol and the skull is exposed. (iii) For lateral ventricular grafts in rats weighing 300–900 g, a large trephine hole (about 1.5 mm by 1.5 mm) centered over the point 1.5 mm anterior and 1.5 mm lateral to the bregma is drilled with a very fine dental burr. Of course, care must be taken to avoid puncturing the sagittal sinus.

II.3. Transplantation method A

The needle used for transplantation consists of a 15-gauge guide cannula, and an 18-gauge spinal tap needle with a close-fitting obdurator (Table II). If necessary the spinal tap needle should be ground and buffed so that it has a short-bevelled tip with no internal of external burrs. The 15-gauge guide cannula should be cut about 10–15 mm shorter than the spinal tap needle.

Procedure: (iv) The dura is cut with a small hypodermic needle. (v) The needle assembly, with the bevel facing laterally, is lowered to coordinates 1.5 mm anterior, 1.5 mm lateral, and 4.0 mm below the dura, and withdrawn to 3.5 mm below the dura. (vi) The inner cannula assembly is then removed, attached to a 1 ml disposable plastic syringe, and filled with Ringer's solution. (vii) The tissue to be transplanted is then very gently manipulated so that it lies in a small bubble of fluid. (viii) The bevel of the needle is then placed against the meniscus of the bubble and the tissue with some fluid is aspirated in a total volume of no more than 20 μl. Care is taken to avoid aspirating air with the tissue. (ix) The needle and syringe assembly is then transported to the guide cannula, being careful to keep the point of the needle oriented downward. (x) With the bevel facing laterally, the assembly is placed into position in the brain by replacing it into the guide cannula. (xi) The 20 μl of fluid is then injected into the ventricle, in several small 'spurts' of about 5 μl (no more than 10 μl) each over the course of 2 min or longer. (xii) The needle is left in place for 2 min and withdrawn. (xiii) The contents of the needle are then inspected to insure that all of the tissue has been administered.

II.4. Transplantation method B

For this method an 18- or 20- gauge deflected-point needle (Table II) or a spinal tap needle is used. The hub of the needle, attached to a 1 ml tuberculin syringe, is attached directly to the electrode carrier with the needle orifice facing laterally. For this purpose we use a David Kopf Instruments universal holder, which must be modified by lengthening the clamping slot.

Procedure: (iv) The tissue to be transplanted is placed in a small bubble of Ringer's solution in a sterile watch glass. (v) The needle and syringe assembly is centered over the proper lateral (1.5 mm) and anterior-posterior (1.5 mm anterior) coordinates. (vi) The entire holder and syringe assembly is removed from the stereotaxic instrument. (vii) The tissue and fluid are aspirated. (viii) The assembly is replaced in the stereotaxic instrument. (ix) The needle tip is lowered to 4.0 mm below the dura and then raised to 3.5 mm. (x) The tissue is expressed in spurts as in Method A. (xi) The needle is then left in place for 2 min and withdrawn. (xii) The contents of the needle are inspected.

Note A

The procedure for ejecting the tissue from the needle always involves injecting small spurts of about 5 μl each, spaced so that the total 20 μl volume is injected over the course of 2 min or longer. The fluid should not be pumped in, nor should the syringe plunger be slowly and gradually depressed, as this will allow the tissues to adhere to the walls of the needle and the grafts will not be expressed into the brain. Practice is essential to develop the necessary 'touch' for injecting 5 μl spurts with a 1 ml syringe. To do this, the syringe and plunger are braced with the thumb and third finger, while the plunger is depressed using the second finger of the opposite hand. Other arrangements, such as infusion pumps, 50 μl syringes, or small glass syringes, are much less satisfactory.

II.5. Post-operative procedures

The trephine hole is wiped with surgical sponge and covered with gelfoam, and the scalp is closed with wound clips. Each animal then receives 0.2 ml of either Bicillin or Flo-Cillin (Table II). It appears to us that the grafts and the animals fare much better post-operatively when penicillin is used routinely. If there is evidence of respiratory distress or if rapid recovery from the anesthesia is desired, the animals are given the respiratory stimulant 3-methyl-3-ethylglutarimide (Sigma Chemical Co., 3% in propylene glycol) i.p., 1.0 ml/kg. The animals are kept in a warm location until they recover from anesthesia.

Note B

Methods have been described for the transplantation of fetal hypothalamic tissue to the third ventricle (Gash and Sladek, 1980) and for transplantation of superior cervical ganglia to the fourth ventricle (Rosenstein and Brightman, 1979). In the studies of Gash and Sladek (1980), tissue was ejected from a 20-gauge spinal tap needle by mechanical pressure from the obdurator. In the studies of Rosenstein and Brightman (1978, 1979), tissue was placed into the fourth ventricle of 6–14-day-old rats by exposing the brain, inserting the tissue through the cisterna magna, and gently manipulating it into position with a glass rod.

III. Results

In our studies, all tissues that have been transplanted into the lateral cerebral ventricles have survived for extended periods of time. In the rat, we have examined homologous brain tissue grafts from several brain regions, adrenal medulla, kidney cortex, and sciatic nerve grafts, as well as allogenic brain

TABLE III

FREQUENCY OF VENTRICULAR GRAFT SURVIVAL IN RATS

(a) *Homologous embryonic substantia nigra grafts*

Duration of survival	Number of rats examined histologically	Percent with surviving graft tissue
3 weeks	52	100*
3 months	13	100
8–10 months	16	100
14–22 months	7	100

(b) *Other homografts*

Tissue	Duration of survival (months)	Number of rats examined histologically	Percent with surviving graft tissue
Embryonic cortex	9–17	7	100
Embryonic tectum	9–17	6	100
Adrenal medulla	5	13	69
Kidney cortex	6	9	100

(c) *Others*

Tissue	Duration of survival (months)	Number of rats examined histologically	Percent with surviving graft tissue
Embryonic brain syngrafts	4–5	2	100
Embryonic brain allografts	2.5–5	5	100
Embryonic brain allografts	6–7	11	73
Adrenal medulla allografts	3	6	67**
Mouse submaxillary gland heterografts	2	3	67

(d) *Graft classification*

Autograft:	donor and host are the same individual.
Syngraft:	donor and host are genetically identical but different individuals.
Homografts (or homologous):	donor and host are of the same species and presumed, but not specifically known to be genetically dissimilar.
Allograft:	donor and host are the same species and are known to be genetically dissimilar.
Heterograft:	donor and host are of different species.

*A single graft was found which showed signs of deterioration such as macrophage accumulation, suggesting that it might eventually have been rejected.
**A few (less than 10) surviving catecholaminergic cells were found in the remaining two animals.

tissue and adrenal medulla, and heterogeneic submaxillary gland grafts. The majority of all types of grafts survived, with varying properties and varying rates of success (Table III).

III.1. Embryonic brain tissue

Embryonic substantia nigra, frontal cortex, and tectum survive consistently when grafted to the lateral ventricle. When examined histologically after periods ranging from three weeks to almost two years, surviving tissue, containing both neurons and glia, has been found in virtually every animal that has been studied (Table III). Of course not all tissue that is implanted survives, and not every *graft* survives, but since multiple grafts are implanted in each rat, some surviving tissue has always been found. For example, in a

recent study of 32 rats with substantia nigra grafts, surviving grafts with numerous catecholamine-containing cells reinnervating the host striatum were found in 27 of the rats (84%) three weeks after implantation. Of the remaining five animals, three (9.4%) had surviving grafts which contained many neurons but only a few (less than 10) catecholaminergic cells, and in two cases (6.3%) the grafts contained catecholaminergic cells but were misplaced, attached to the corpus callosum or to the septum.

These grafts usually have a healthy appearance, and do not contain debris, autofluorescent macrophages, or excessive accumulations of lipofuscin at any of the survival times examined. The grafts become well vascularized.

Tissues grafted to the ventricles usually become tightly adherent to the walls of the ventricle, in our studies usually to the striatum, although the surgery can be arranged so that the grafts adhere to other periventricular structures. In a few cases, however, the grafts or parts of the grafts are loosely attached to the host brain through fine fiber bridges, and in these cases the grafts are easily torn away from the host brain during sectioning.

III.2. Adrenal medulla

These grafts are found to survive in most, but not in all, animals. Overall, substantial amounts of surviving tissue have been found in approximately two-thirds of the animals that have been examined, and at least a few surviving cells were found in about 80% of the animals (Table III). There is also an obvious difference in the general appearance of adrenal medulla and embryonic brain tissue grafts, in that the adrenal medulla grafts are not as well integrated with the host brain tissue. These grafts invariably contain some debris and autofluorescent macrophages, and the border between the graft and the host brain can always be precisely discerned.

III.3. Kidney cortex

Kidney cortex grafts were implanted with the intention of influencing the subfornical organ by secreting renin into the cerebrospinal fluid. These grafts invariably grew quite large and survived for extended periods, but also contained large necrotic areas which comprised between 25 and 50% of the total graft volume. The non-necrotic parts of the grafts were generally in healthy condition.

III.4. Mouse submaxillary gland

Male mouse submaxillary gland pieces were transplanted to the rat lateral ventricles for the purpose of secreting nerve growth factor. These grafts were found to have survived by histological criteria (Table III) when examined after approximately two months (Freed, 1983).

III.5. Sub-human primates

In monkeys, we have been relatively unsuccessful in obtaining survival of intraventricular brain tissue homografts and adrenal medulla autografts. For example, in recent studies of adrenal medulla autografts in rhesus monkeys, tissue was implanted into both the lateral ventricles and the body of the caudate of several animals. Although the grafts in the brain parenchyma survived to varying degrees, none of the tissue that was transplanted to the ventricles was found (Morihisa et al., 1984). Since the tissue was autologous, and also since the intraparenchymal grafts survived, the intraventricular grafts could not have been rejected. It is suspected, therefore, that the failure of these grafts was due to a lack of adhesion of the grafts to the walls of the ventricle and a consequent failure of the brain to vascularize the grafted tissues.

IV. Discussion and Conclusions

The primary advantage of the ventricular system as a site for tissue transplantation is that it provides an area into which tissue can be placed essentially with very little damage to the host brain parenchyma. Intracortical or intraparenchymal grafts, as well as grafts into cortical cavities, inevitably damage or disrupt some host brain tissue. Even the minimal damage produced by intraparenchymal grafts of dissociated cells could become a significant factor under some circumstances, for example if it became necessary to graft tissue into numerous sites. Even fairly large intraventricular grafts, however, do not appear to damage the host brain, except in the cortex along the needle penetration tract. The cerebrospinal fluid appears to provide a good medium for initial maintenance of various types of tissues, and intraventricular grafts can grow to become fairly large. As suggested by Rosenstein and Brightman (1978, 1979), the intraventricular grafting technique provides a means of investigating interactions between transplanted tissues and intact, undamaged brain surfaces.

On the other hand, the ventricular system provides access only to periven-

tricular structures. Brain nuclei which do not come into close contact with the ventricular system (for example, the cerebral cortex, the amygdala, or the substantia nigra) probably cannot be innervated by grafts in the cerebral ventricles. In particular, the caudate-putamen of the rat contacts the lateral ventricle but extends laterally away from the ventricle for more than 3 mm. Embryonic substantia nigra grafts in the lateral ventricle reinnervate only the periventricular parts of the caudate-putamen. Those parts of the caudate-putamen which are remote from the ventricular system are not reinnervated, even though graft-derived fibers have entered the structure. On the other hand, when the goal is to employ tissue transplantation to influence parts of the brain which are immediately adjacent to the ventricles, intraventricular grafting may be an ideal technique.

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